

# Familial advanced sleep-phase syndrome: A short-period circadian rhythm variant in humans

CHRISTOPHER R. JONES<sup>1,2</sup>, SCOTT S. CAMPBELL<sup>9</sup>, STEPHANIE E. ZONE<sup>3</sup>, FRED COOPER<sup>4</sup>,  
ALISON DE SANO<sup>3</sup>, PATRICIA J. MURPHY<sup>9</sup>, BRYAN JONES<sup>5</sup>,  
LAURA CZAJKOWSKI<sup>6</sup> & LOUIS J. PTÁČEK<sup>1,3,7,8</sup>

<sup>1</sup>Department of Neurology, <sup>2</sup>University Hospital Sleep Disorders Center, <sup>3</sup>Howard Hughes Medical Institute,

<sup>4</sup>Department of Management; David Eccles School of Business, <sup>5</sup>Department of Physiology,

<sup>6</sup>Department of Psychiatry, <sup>7</sup>Department of Human Genetics,

<sup>8</sup>Program in Neuroscience, University of Utah, Salt Lake City, Utah 84112, USA

<sup>9</sup>Department of Psychiatry, Weill Medical College of Cornell University, White Plains, New York 10605, USA

Correspondence should be addressed to L.J.P.

Biological circadian clocks oscillate with an approximately 24-hour period, are ubiquitous, and presumably confer a selective advantage by anticipating the transitions between day and night. The circadian rhythms of sleep, melatonin secretion and body core temperature are thought to be generated by the suprachiasmatic nucleus of the hypothalamus, the anatomic locus of the mammalian circadian clock<sup>1,2</sup>. Autosomal semi-dominant mutations in rodents with fast or slow biological clocks (that is, short or long endogenous period lengths;  $\tau$ ) are associated with phase-advanced or delayed sleep-wake rhythms, respectively. These models predict the existence of familial human circadian rhythm variants<sup>3,4</sup> but none of the human circadian rhythm disorders are known to have a familial tendency<sup>5</sup>. Although a slight 'morning lark' tendency is common, individuals with a large and disabling sleep phase-advance are rare. This disorder, advanced sleep-phase syndrome, is characterized by very early sleep onset and offset; only two cases are reported in young adults<sup>6,7</sup>. Here we describe three kindreds with a profound phase advance of the sleep-wake, melatonin and temperature rhythms associated with a very short  $\tau$ . The trait segregates as an autosomal dominant with high penetrance. These kindreds represent a well-characterized familial circadian rhythm variant in humans and provide a unique opportunity for genetic analysis of human circadian physiology.

The first advanced sleep-phase syndrome (ASPS) patient in this study presented to our sleep center with disabling early evening sleepiness and early morning awakening. Because she recognized a similar trait in some family members, we evaluated consenting relatives from her extended family and from two additional families that were subsequently identified. A structured interview with each participant focused on the underlying preferred sleep schedule in the absence of psychosocial factors that would delay or advance sleep phase. Individuals were considered 'affected' if they described a life-long, stable pattern of early sleep onset and offset and met strict classification criteria. Individuals were considered 'unaffected' if they described an unambiguously normal or delayed sleep-wake schedule and 'unknown' if they did not meet either affected or unaffected criteria. We administered the Horne-Östberg questionnaire<sup>8</sup> (a validated measure of 'morning lark' or 'night owl' tendency).

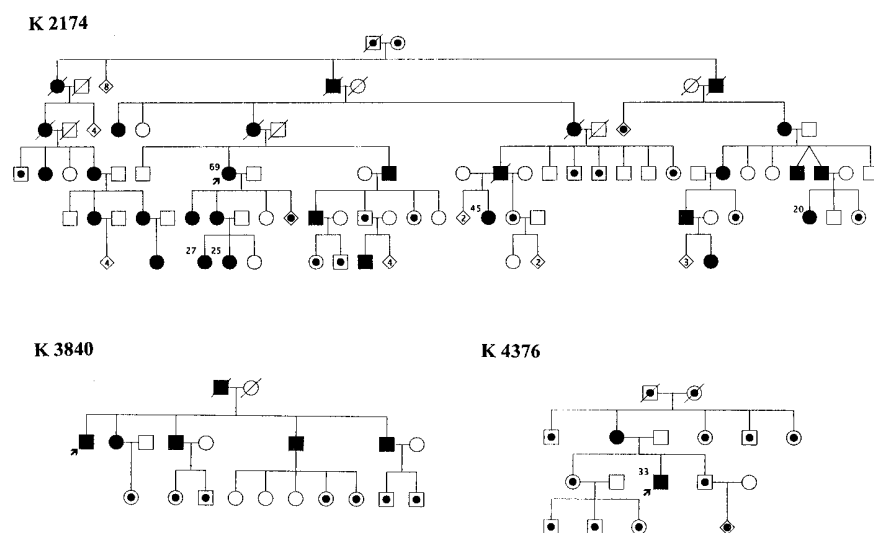
Using our strict classification criteria, we identified 29 people with familial ASPS (FASPS) and 46 unaffected people. ASPS segre-

gates as a highly penetrant autosomal dominant trait in these families (Fig. 1). All three families are of Northern European descent (ancestors of kindreds 2174 and 3840 were from the British Isles). The youngest affected subject was 8 years old. Most FASPS subjects knew they were obligate 'morning larks' by 30 years of age. The Horne-Östberg scores were consistent with our classification scheme: FASPS patients,  $76.2 \pm 5.6$  ( $n = 14$ ); unaffected relatives,  $60.5 \pm 6.8$  ( $n = 12$ ) ( $P < 0.0005$ ) (ref. 8). The Horne-Östberg scores of first-degree relatives (unaffected and unknown) of affected individuals ( $63.7 \pm 7.1$ ;  $n = 24$ ) were higher than those of 'marry-in' spouses and unrelated control subjects ( $55.2 \pm 11.0$ ;  $n = 33$ ). Among first-degree relatives of affected individuals, there was a nonsignificant trend ( $P = 0.068$ ) towards higher scores for unknown subjects ( $66.8 \pm 6.2$ ;  $n = 12$ ) than for unaffected subjects ( $60.5 \pm 6.8$ ;  $n = 12$ ). This is consistent with our conservative classification scheme, because we expect to have classified some FASPS gene carriers as unknown.

Six FASPS subjects (20–69, average,  $37 \pm 18$  years) and six unrelated gender- and age-matched ( $\pm 6$  years) control subjects were admitted for inpatient study. All participants were found to be generally healthy by medical history and physical exam. Scores from both FASPS and control groups on the Beck depression inventory were in the range of 'minimal depression'<sup>9</sup>. However, screening for affective disorder by a psychologist showed major depression in one and mild depression in another two FASPS subjects.

The 12 inpatient subjects were admitted in the early afternoon for two consecutive nights of polysomnographic assessment of sleep phase and sleep quality, followed each morning by a multiple sleep latency test. The polysomnography estimates sleepiness by measuring the latency to sleep onset in multiple nap trials during the day. The polysomnographic and multiple sleep latency tests were recorded and scored according to standard procedures<sup>10–12</sup>. No common sedatives or stimulants were detected in the urine of subjects after the first night of polysomnographic recording.

Polysomnographic measures of sleep phase including the time of sleep onset, sleep offset, first slow wave sleep and first rapid eye movement sleep were advanced by almost 4 hours in FASPS subjects compared with those of control subjects (Table 1). Polysomnographic measures of sleep quality and quantity were within normal limits for both FASPS ( $n = 5$ ) and control ( $n = 6$ ) subjects: total sleep time (minutes),  $425.3 \pm 59.92$  and  $445.42 \pm$



**Fig. 1** Pedigrees of three FASPS kindreds. Circles, males; squares, females. Filled symbols, affected individuals; open symbols, unaffected subjects; symbols with central dots, individuals of unknown phenotype; diamonds, sibships of children with unknown phenotype (number in diamond, sibship size). Number at upper left of symbol, inpatient participant identifier (age in years). Arrows, probands.

ical assessment that they were not sleep phase-delayed.

There was also a profound qualitative difference between groups. People with conventional sleep schedules tend to stay up later and wake up later when on vacation. In contrast, FASPS subjects tend to fall asleep even earlier and also to wake up earlier during vacation, consistent with their substantial tendency towards sleep-phase advance (data not shown).

83.48; % stage 1 sleep,  $11.72 \pm 3.79$  and  $12.83 \pm 4.37$ ; % rapid eye movement sleep,  $20.08 \pm 3.72$  and  $21.00 \pm 7.58$ ; and % slow wave sleep,  $10.30 \pm 7.02$  and  $10.44 \pm 3.59$ . One FASPS subject had evidence of moderate obstructive sleep apnea and one control subject had periodic limb movements in sleep with microarousals. None of the multiple sleep latency test results from control or FASPS subjects were indicative of narcolepsy or other cause of excessive daytime sleepiness.

We determined circadian phase using plasma melatonin and body core temperature measurements<sup>13</sup>. The melatonin and temperature rhythms were both phase-advanced by 3–4 hours in FASPS subjects compared with control subjects (Table 1).

To eliminate the possibility of sleep deprivation or self-imposed unconventional sleep-wake schedules, subjects kept 'sleep logs' at home for 1 week before admission to and for 2 weeks after leaving the Clinical Research Center. There was no consistent seasonal bias for date of inpatient study in FASPS compared with control subjects.

Activity levels (actigraphy) were also recorded during the inpatient stay and for 3 weeks after subjects went home. The phase advance of self-reported sleep times in FASPS and control subjects was consistent with ambulatory actigraphy and sleep log data. By all three measures, FASPS patients were sleep phase-advanced by 3–4 hours compared with control subjects (data not shown). The large difference in Horne-Östberg scores for FASPS patients ( $77 \pm 6.7$ ;  $n = 5$ ) and control subjects ( $48.2 \pm 4.6$ ;  $n = 6$ ) ( $P = 0.006$ ) is consistent with a phase advance of this large magnitude. The average Horne-Östberg score of 48.2 for the control subjects also supports our sleep log, actigraphy and clin-

We studied one 69-year-old subject in a time isolation facility to determine the endogenous period of her circadian clock. We determined sleep-wake and temperature data during the time isolation study (Fig. 2). Periodograms showed a very short  $\tau$  (23.3 hours) for both rhythms compared with those of a sex- and age-matched control subject (24.2 hours) and with estimates of 24.0 to 24.5 hours in other studies<sup>14</sup>.

Our results define a hereditary circadian rhythm variant in humans associated with a short endogenous period. The clinical histories, sleep logs and ambulatory actigraphy patterns of FASPS subjects demonstrated a significant phase advance of the sleep-wake rhythm in normal life settings relative not only to our control subjects, but also to sleep-wake schedules widely held to be conventional and to published values for sleep-wake schedules<sup>8</sup>. Inpatient recordings confirmed the early sleep phase and showed that the melatonin and temperature rhythms were also advanced. FASPS subjects tended to fall asleep during solar clock times that correspond to the 'maintenance of wakefulness zone' in conventional sleepers<sup>15,16</sup>. Similarly, FASPS subjects tended to wake up during solar clock times that correspond to the circadian peak of sleepiness in conventional sleepers<sup>15,16</sup>.

To our knowledge, no well-characterized monogenic circadian rhythm variant has previously been reported in humans. Furthermore, a profoundly advanced sleep-wake rhythm has been thought to be exceedingly rare in healthy, young, non-depressed adults<sup>5</sup>. However, in the families reported here, there is a clear autosomal dominant transmission of profound sleep phase advance, indicating that ASPS in the young is more common than previously thought.

Several observations support the assertion that FASPS is a genetic trait and not a learned habit. Siblings in these kindreds were often widely divergent for morning, evening or conventional sleep-wake preference; moreover, the onset of the phenotype is often after young adults are living independently. Thus, parental or cultural factors are not a reasonable explanation for this robust and stable phase advance. This is consistent with other evidence that 'morningness-eveningness' is stable despite variable social and environmental factors<sup>17</sup>. Phenotypically, FASPS

**Table 1** Phase markers of overt rhythms

	Control ( $n = 6$ ) Mean $\pm$ s.d.	FASPS( $n = 6$ ) Mean $\pm$ s.d.	Difference (hours:minutes)	P value
Sleep Onset	23:10 $\pm$ 0:40	19:25 $\pm$ 1:44	3:45	< 0.0005
Sleep Offset <sup>a</sup>	07:44 $\pm$ 1:13	04:18 $\pm$ 2:00	3:26	< 0.0005
1st Slow Wave Sleep	23:55 $\pm$ 1:17	20:14 $\pm$ 2:35	3:41	0.002
1st REM <sup>a</sup>	00:55 $\pm$ 1:29	21:16 $\pm$ 2:25	3:39	< 0.0005
DLMO	21:21 $\pm$ 0:28	17:31 $\pm$ 1:49	3:50	< 0.0005
Temp Nadir <sup>b</sup>	03:35 $\pm$ 1:33	23:22 $\pm$ 2:55	4:13	0.002

<sup>a</sup> $n = 5$  for FASPS only. <sup>b</sup> $n = 5$  for control and FASPS. Data include both nights of study. REM, rapid eye movement; DLMO, dim-light melatonin onset; Temp, temperature.

## ARTICLES

is similar to the short period of the  $\tau$  mutant hamster<sup>3</sup>. The existence of such mutants in rodents predicts that analogous traits may occur in humans.

Early sleep onset can result from pathologic sleepiness of any cause, such as narcolepsy, obstructive sleep apnea or 'restless legs' syndrome. These disorders were not found in the FASPS group. Early morning awakening is a symptom of major depression. However, depression is not an explanation for our findings, as several of our more profoundly sleep-phase advanced subjects were free of this affective disorder. The sleep-wake rhythm is thought to be phase-advanced in the elderly<sup>18,19</sup>, but our FASPS subjects were affected at an early age.

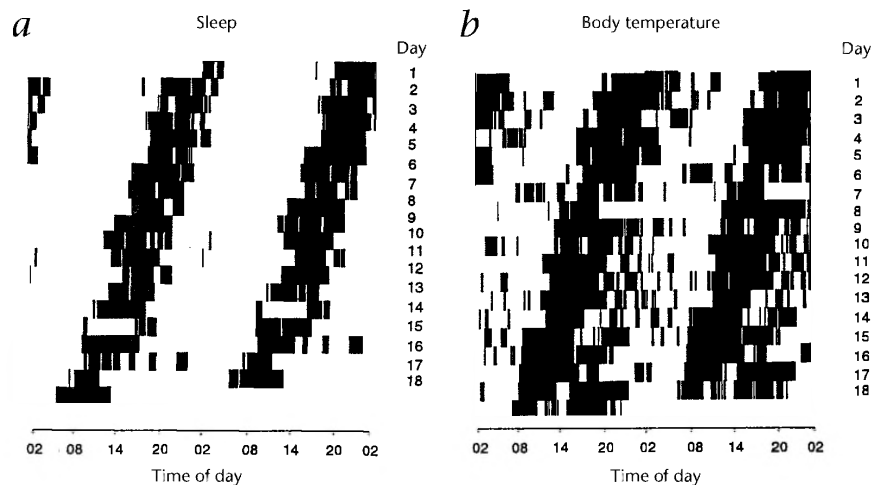
The range of sleep preferences in humans covers the spectrum from 'morning larks' to 'night owls'. A study of 220 twin pairs using the Horne-Östberg questionnaire showed that much of morning and evening preference is heritable<sup>20</sup>. One allele from a polymorphism in the human *clock* gene was found to predict a small delay in the preferred time for sleep<sup>21</sup>. However, the mendelian inheritance pattern and the large magnitude of the phase advance reported here indicate that FASPS is distinct from the more common and heritable 'morning-type person'. Therefore, the allele causing FASPS may prove to have a quantitatively larger effect on clock function than the more common genetic variations that influence morning or evening preference.

Theoretically, even a small shortening or lengthening of  $\tau$  can result in a much earlier or later onset of sleep, respectively, relative to the light-dark cycle<sup>22</sup>. Empiric evidence for this is limited in humans<sup>23</sup>, but the heterozygous  $\tau$  mutant golden hamster does have a short free-running period (22 hours) and activity onsets that are about 4 hours early in a 24-hour light-dark cycle<sup>3</sup>. Therefore to our knowledge the 23.3-hour  $\tau$  described here represents not only the shortest  $\tau$  reported in humans, but also the first reported change of  $\tau$  in the expected direction in a person with an abnormal sleep phase.

Clues to the functional role of human clock genes are provided by the molecular biology of clocks in model systems<sup>24</sup>. Humans seem to have the same fundamental clock mechanisms as *Drosophila* and mice. However, diurnal humans must have some subtle differences in clock entrainment characteristics and output coupling mechanisms compared with those of nocturnal rodents and insects. Therefore, genetic clock variants such as FASPS will be valuable in confirming basic clock mechanisms and furthering our understanding of circadian physiology in humans.

## Methods

**Diagnosis and classification criteria.** Subjects signed a consent form approved by the Institutional Review Board at the University of Utah. Individuals were considered to have ASPS if the subject was capable of falling asleep before 20:30 and waking before 05:30 local time daily and throughout the year, if demands and psychosocial preferences that compete with bedtime and sleep were eliminated; if there was only one major sleep period per 24-hour day; if the onset was before 40 years of age; and if the ASPS did not develop within 3 months of traumatic brain injury and was not maintained by morning stimulant drugs or evening sedative



**Fig. 2** Free-running period of the circadian sleep-wake and temperature rhythms in one FASPS subject. Sleep-wake (*a*) and body core temperature (*b*) rhythms of a 69-year-old female from kindred 2174 (Fig. 1), studied in time isolation for 18 d. Data are double-plotted. *a*, Sleep data (filled bars) are derived from polygraphically-recorded sleep, scored using standard criteria<sup>11</sup>. *b*, Temperature plot shows body temperature below the daily mean (filled bars). Chi-squared periodogram analysis showed a free-running period of 23.3 h for both variables during the 18-day recording interval.

drugs, early morning work start-time, other early morning psychosocial demands or self-imposed early morning bright light. Individuals were considered unaffected if they preferred to go to sleep after 21:30 and to wake after 06:30 in the absence of any social or work responsibilities; and if this preference was stable from before the age of 40 and was independent of any stimulant or sedative drugs. Individuals not meeting either 'affected' or 'unaffected' criteria were considered to be of 'unknown' phenotype.

**Actigraphy.** During the inpatient stay, recordings were made with 1-minute epochs, using the Mini-Logger Series 2000 wristband activity dual-axis sensor (Mini-Mitter, Sun River, Oregon). Outpatient actigraphy data was collected for 3–4 weeks immediately after patients were discharged from the Clinical Research Center, using actiwatches (provided by J. Takahashi, Cambridge Neurotechnology, Cambridge, UK) with coincident sleep logs on which patients recorded their subjective sleep and wake times. For these recordings, 5-minute epochs were recorded over a period of 22–30 d. Activity amplitude was determined by the number of switch closures during each measurement interval. Actigraph data was analyzed for average activity offset and onset times using the Mini-Mitter algorithm. The time between the activity offset and onset represents the longest period of inactivity (presumed sleep time) during a 24-hour period. Activity offset was defined as the first of 12 consecutive epochs during which a maximum of 1 epoch contained a nonzero value. Accordingly, activity onset was defined as the first of 12 consecutive epochs during which a maximum of 1 epoch contained a zero value. Time values from the sleep logs were rounded to the nearest 15-minute interval. These and subsequent determinations of statistical significance were based on two-tailed Mann-Whitney tests.

**Polysomnography and multiple sleep latency tests.** Polysomnography included three-channel electroencephalogram, two-channel electro-oculogram, submental surface electromyogram, anterior tibialis surface electromyogram, rhythm electrocardiogram, tracheal microphone, ribcage and abdominal peizo-belts, nasal-oral 'thermistor' and Nellcor N-200 finger pulse oximetry. All signals were amplified, filtered, sampled at 256 Hz, digitized and then stored on computer disc at approximately 70 Hz using the Nellcor Puritan Bennett Company (Ottawa, Ontario, Canada) 'Sandman' software (version 2.3) system on a personal computer. Sleep stages were scored using standard criteria<sup>11</sup> and 30-second epochs. Nighttime sleep onset was defined as the beginning of the first of five consecutive epochs of sleep. Slow-wave sleep and rapid eye movement



sleep latencies were defined as the time from sleep onset to the first epoch of stage three or four sleep and rapid eye movement sleep, respectively. Sleep stage percentages were calculated using the sleep period time as the denominator. The measures of sleep were similar between nights one and two, and values for the two nights were averaged. Apneas, hypopneas, micro-arousals and periodic leg movements were scored according to standard criteria by a diplomate of the American Board of Sleep Medicine (C.R.J.). Bedtime and morning wake times were self-selected by all subjects. Multiple sleep latency tests included four to five nap opportunities (determined by the time of patient's morning awakening) and were scored according to standard clinical criteria<sup>12</sup>. The multiple sleep latency tests started at 06:00 for the subjects with FASPS and at 09:00 for the control subjects. The mean sleep latencies were similar for both days and were therefore averaged.

**Temperature and melatonin measurements.** Dim ambient room light (less than 50 lux) was enforced after 16:00 and 18:00 until final morning awakening for the FASPS and control subjects, respectively, to avoid 'masking' the expected time of melatonin onset<sup>13</sup>. Bedtime and morning wake times were self-selected by both FASPS and control subjects. During the day, normal light exposure was allowed. Visiting hours and telephone call-in times were restricted to the daytime hours between multiple sleep latency test naps.

Blood samples for plasma melatonin were drawn from an indwelling forearm catheter. Blood was drawn every half-hour between 16:00 and 24:00 for the FASPS subjects and between 18:00 and 02:00 for the control subjects and at 2-hour intervals for the remainder of the day. Samples were centrifuged, separated, and frozen at -20 °C.

Plasma melatonin levels were measured using the Buhlmann melatonin double antibody radioimmunoassay kit (ALPCO, Windham, New Hampshire) with the Kennaway G-280 antibody against melatonin (assays were done by A. Clemons, Oregon Health Sciences University). Plasma samples and known calibration samples were initially extracted using C18 reversed-phase extraction columns and eluted with methanol. Data were analyzed using Beckman ImmunoFit EIA/RIA data-reduction software on a cubic spline curve fit. Samples with initial values greater than 100 pg/ml were diluted and reassayed. The dim-light melatonin onset was defined as the clock time at which the melatonin value reached 10 pg/ml (ref. 13).

Core temperature monitoring was accomplished using a rectal temperature probe. A plastic-coated thermocouple inserted approximately 5–10 cm into the rectum provided continuous temperature measurements throughout the subject's inpatient stay. The data were sampled and stored every 1 minute on a Mini-Mitter® data logger and downloaded to a personal computer for graphical output. The nadirs of the manifest (masked) temperature rhythms were determined by establishing the minima after polynomial fitting of the temperature data. In determining an individual nadir, only data from sleep onset through sleep offset was used for model fitting. Linear and curvilinear regression and logarithmic models for determining temperature minima yielded similar results.

**Free-running period measurement.** Immediately after a 3-day entrainment period on a 24-hour sleep-wake schedule, this 69-year-old subject was studied for 18 d in a laboratory apartment without any cues to time of day. The subject was instructed to eat and sleep whenever she wished, except that she was requested not to take naps. During her waking hours, she was permitted leisure activities in less than 150 lux ambient light. EEG and body core temperature were recorded continuously throughout her 3-week laboratory stay. Body temperature was measured by an indwelling rectal 'thermistor' sampled every 2 min throughout the isolation period. The

sleep and wakefulness scoring by standard criteria<sup>11</sup> demonstrated normal sleep architecture and sleep quality.

#### Acknowledgments

The authors thank A.J. Lewy, who provided discussions and comments, and A. Dean and T. Schenkenberg for critical reading of this manuscript. This investigation was supported by NIH grant HL/HD 59596 (L.J.P.) and Public Health Service research grant M01-RR00064 from the National Center for Research Resources.

RECEIVED 4 MAY; ACCEPTED 28 JUNE 1999

- Shanahan, T.L. & Czeisler, C.A. Light exposure induces equivalent phase shifts of the endogenous circadian rhythms of circulating plasma melatonin and core body temperature in men. *J. Clin. Endocrinol. Metab.* **73**, 27–35 (1991).
- Czeisler, C.A., Weitzman, E., Moore-Ede, M.C., Zimmerman, J.C. & Knauer, R.S. Human sleep: its duration and organization depend on its circadian phase. *Science* **210**, 1264–1267 (1980).
- Ralph, M.R. & Menaker, M. A mutation of the circadian system in golden hamsters. *Science* **241**, 1225–1227 (1988).
- Vitaterna, M.H. et al. Mutagenesis and mapping of a mouse gene, Clock, essential for circadian behavior. *Science* **264**, 719–725 (1994).
- American Sleep Disorders Association in *International Classification of Sleep Disorders: (Revised): Diagnostic and Coding Manual* (Chairman, Thorpy, M.J.) 133–136 (Rochester, Minnesota, 1997).
- Billard, M. et al. A case of advanced-sleep phase syndrome. *Sleep Res.* **22**, 109 (1993).
- Singer, C.M. & Lewy, A.J. Case report: Use of the dim light melatonin onset in the treatment of ASPS with bright light. *Sleep Res.* **18**, 445 (1989).
- Horne, J.A. & Östberg, O. A self-assessment questionnaire to determine morningness-eveningness in human circadian rhythms. *Int. J. Chronobiol.* **4**, 97–110 (1976).
- Beck, A.T. *The Beck Depression Inventory* (BDI-II) 1–2 (Harcourt Brace & Company, San Antonio, Texas, 1978).
- Keenan, S.A. in *Sleep Disorders Medicine: Basic Science, Technical Considerations and Clinical Aspects* (ed. Chockroverty S) 79–94 (Butterworth-Heinemann, Boston, 1994).
- Rechtschaffen, A. & Kales, A. in *A Manual of Standardized Terminology, Techniques and Scoring System for Sleep Stages of Human Subjects* (eds. Rechtschaffen, A. & Kales, A.) 1–12 (UCLA Brain Information Service/Brain Research Institute, Los Angeles, 1968).
- Carskadon, M.A. et al. Guidelines for the multiple sleep latency test (MSLT): a standard measure of sleepiness. *Sleep* **9**, 519–524 (1986).
- Lewy, A.J. & Sack, R.L. The dim light melatonin onset as a marker for circadian phase position. *Chronobiol. Int.* **6**, 93–102 (1989).
- Campbell, S.S., Dawson, D. & Zullig, J. When the human circadian system is caught napping: evidence for endogenous rhythms close to 24 hours. *Sleep* **16**, 638–640 (1993).
- Carskadon, M.A. & Dement, W.C. Sleep studies on a 90-minute day. *Electroencephalogr. Clin. Neurophysiol.* **39**, 145–155 (1975).
- Lavie, P. Ultrashort sleep-waking schedule. III. 'Gates' and 'forbidden zones' for sleep. *Electroencephalogr. Clin. Neurophysiol.* **63**, 414–425 (1986).
- Greenwood, K.M. Long-term stability and psychometric properties of the composite scale of morningness. *Ergonomics* **37**, 377–383 (1994).
- Campbell, S.S., Gillin, J.C., Kripke, D.F., Erikson, P. & Clopton, P. Gender differences in the circadian temperature rhythms of healthy elderly subjects: relationships to sleep quality. *Sleep* **12**, 529–536 (1989).
- Haimov, I. & Lavie, P. Circadian characteristics of sleep propensity function in healthy elderly: a comparison with young adults. *Sleep* **20**, 294–300 (1997).
- Drennan, M.D., Selby, J., Kripke, D.F., Kelsoe, J. & Gillin, J.C. Morningness/eveningness is heritable. *Soc. Neurosci. Abstr.* **18**, 196 (1992).
- Katzenberg, D. et al. A CLOCK polymorphism associated with human diurnal preference. *Sleep* **21**, 569–576 (1998).
- Klerman, E.B., Dijk, D.J., Kronauer, R.E. & Czeisler, C.A. Simulations of flight effects on the human circadian pacemaker: implications for assessment of intrinsic period. *Am. J. Physiol.* **270**, R271–R282 (1996).
- Duffy, J.F., Rimmer, D.W., Silva, E.J. & Czeisler, C.A. Correlation of intrinsic circadian period with morningness-eveningness in young men. *Sleep* **22**, 592 (1999).
- Darlington, T.K. et al. Closing the circadian loop: CLOCK-induced transcription of its own inhibitors per and tim. *Science* **280**, 1599–1603 (1998).